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Home Air Nicotine Levels and Urinary Cotinine Excretion in Preschool Children[1-4]

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SUMMARY We examined the extent of correlation between home air nicotine levels and urine cotinine/creatinine ratios (CCR) in 27 children who attended a research day care program where they were not exposed to environmental tobacco smoke (ETS) during the daytime hours. Average concentrations of nicotine in home air were determined by active air sampling during the evening and night hours on 2 consecutive days. Urine samples for cotinine and creatinine determinations were collected before, during, and after the two sampling periods. In addition, four sequential weekly urine samples for CCR were obtained from study children to determine the extent to which single determinations of CCR were representative for individual children. Fifteen children resided in homes with smokers, and 12 did not. Urine CCR consistently distinguished most exposed and unexposed children. However, three exposed children had urine CCRs that clustered routinely around the criterion CCR (30 ng/mg cotinine-creatinine) that best distinguished exposed and unexposed children. In children exposed to ETS in the home, there was a significant correlation between average home air nicotine levels and the average logarithm of urine CCR the two mornings after the home air monitoring periods ($r = 0.66$; $p = 0.006$). In study children, urine CCRs were remarkably stable over the 1-month observation period. Rank correlation coefficients for sequential weekly determinations of CCR were consistently greater than $r = 0.88$; $p < 0.0001$.

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Introduction

Measurement of cotinine concentrations in body fluids has received recent scrutiny for its usefulness as an indicator of tobacco smoke exposure in adults and children (1-13). Average levels of cotinine in serum, saliva, and urine differ significantly for unexposed individuals, those exposed passively to environmental tobacco smoke (ETS), and active smokers. There remain questions, however, regarding the extent to which cotinine levels in body fluids are quantitatively indicative of the intensity of ETS exposure among passive smokers. Lack of this knowledge and the absence of information regarding the extent to which single cotinine determinations are representative for individuals has limited the usefulness of cotinine measurements in epidemiologic studies of the health effects of passive smoking.

In the present study, we examined the relationship between levels of nicotine in the home air and levels of cotinine in urine of 27 children between 11 months and 5 yr of age. In addition, we obtained a midweek urine sample from study children for cotinine determination each of 4 consecutive weeks to examine the stability over time of this biologic marker of ETS exposure.

Methods

The children studied attended the research day care program of the Frank Porter Graham Child Development Center in Chapel Hill, North Carolina. The respiratory disease research conducted in this population has been described previously (14-16). Children start attending the Center between 6 wk and 3 months of age and are followed continuously to the age of kindergarten entry. They attend the day care program 8 h/day, 5 days/wk and return home each evening and on weekends. Smoking is not permitted in child care areas of the day care center or in vans that are used to transport children to and from the day care center. Twenty-seven children participated in the study: 15 from homes with smokers and 12 from homes without smokers. Three pairs of siblings were studied: two pairs from smoking homes and one pair from a nonsmoking home. The study population consisted of 15 males and 12 females; 14 black and 13 white children. Their ages ranged from 11 months to 5 yr.

Measurement of the Extent of Tobacco Smoking in the Home

Three methods were used to estimate the concentration of tobacco smoke present in home air. (*1*) A questionnaire was administered with questions that identified the persons who smoked in the home and how many cigarettes were smoked. (*2*) Parents were asked to save the unsmoked portions of all cigarettes smoked in the home over 2 consecutive monitoring days. Parents were also asked to save the remnants of cigarettes smoked in their cars while riding with their children. Separate cigarette collections were made for each study day. (*3*) Active home air sampling for nicotine was performed simultaneously with cigarette remnant collections. Air sampling pumps (Model 520; Gilian Co., Wayne, NJ) operating at 15 L/min were employed with Andersen sampling heads (Particle Fractionating Sampler; Andersen Co., Atlanta, GA). Teflon-coated glass fiber filters (75 mm, T60A20; Pallflex, Putnam, CT) impregnated with aqueous 5% sodium bisulfate were used for nicotine trapping (17). The pumps operated from 5:00 P.M. to 7:00 A.M. for each of the 2 days of monitoring. Separate air nicotine determinations were made for each study day. Sampling heads were kept in the child's main activity room during the evening hours, then moved to the child's bedroom at bedtime.

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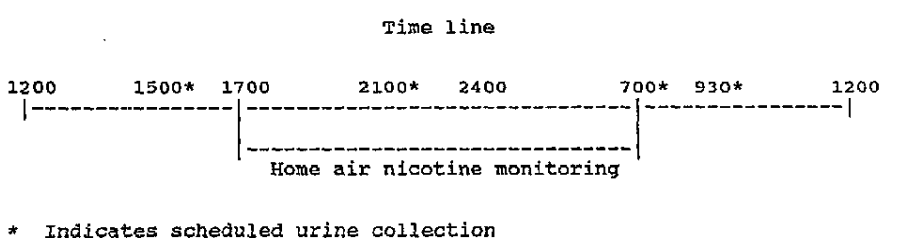

Fig. 1. Home air monitoring and urine collection schedule: time line for both study days 1 and 2.

Collection of Urine Specimens for Cotinine Determinations

Urine specimens were obtained before, during, and at the end of the period of home air nicotine monitoring (figure 1). Children who were not toilet-trained (n = 9) did not have urine specimens collected at home. These children had the 3:00 P.M. and midmorning urine specimens specified by the protocol collected while they were at the day care center. In addition, urine specimens for cotinine determination were obtained on four consecutive Thursdays after the home air monitoring period.

Measurement of Nicotine and Cotinine

Nicotine was eluted from sodium bisulfate-impregnated filters into ethanol and subsequently quantitated by gas chromatography using described methods (17). Concentrations of cotinine in urine samples were determined by competitive-inhibition radioimmunoassay using rabbit cotinine antiserum and tritiated cotinine (purchased from Helen VanVunakis, Brandeis University) (18). To adjust for urine dilution, urine cotinine concentrations were standardized to creatinine concentration and expressed as cotinine/creatinine ratios (CCR).

Results

Comparability of Estimates of Quantity of Tobacco Smoked in Home and Classification of Exposure Status

Except for one instance, there was agreement among the three measures of exposure employed: questionnaire, cigarette remnant collection, and active home air nicotine monitoring. Figure 2 shows the distribution of two consecutive home air nicotine determinations in homes where parents did or did not save cigarette remnants. Average air nicotine concentrations in homes where cigarette remnants were saved were consistently greater than 0.5 $\mu g/m^3$; all children from these homes were considered exposed to ETS in the home. One family that reported smoking in the home in questionnaire responses saved no cigarette remnants. Average concentrations of nicotine in home air were greater than 0.5 $\mu g/m^3$ on both monitoring days; therefore, this child was classified as exposed to ETS in the home. Otherwise, children who lived in homes where no cigarette remnants were saved were classified as not exposed to ETS in the home. The average concentration of air nicotine in the homes of unexposed children was 0.34 $\mu g/m^3$ (SEM, 0.07 $\mu g/m^3$) and in the homes of exposed children was 3.74 $\mu g/m^3$ (SEM, 0.52 $\mu g/m^3$).

Accuracy of Exposure Assignment Using Midmorning Urine Cotinine Determinations

The study protocol specified that midmorning urine specimens for cotinine be collected from study participants 6 times over a 5-wk period (two consecutive mornings after active home air nicotine monitoring and four consecutive Thursdays thereafter). A total of 153 of 162 (94%) of these urine specimens were successfully obtained and assayed for cotinine and creatinine concentrations. The distribution of CCR for these specimens is plotted in figure 3 in relation to exposure status. A urine CCR criterion level of 30 ng/mg resulted in the highest rate of agreement in exposure assignment. Sixty-six of 69 (95%) midmorning urine specimens obtained from 12 unexposed children had CCRs $\leq$ 30 ng/mg (first column, figure 3). Urine specimens from 12 of 15 exposed children consistently (63 of 67; 94%) contained greater than 30 ng cotinine/mg creatinine (second column, figure 3). However, three exposed children consistently had urine CCRs that clustered near the 30 ng/mg breakpoint (13 of 18 = 72% of urine specimens tested had CCR < 30 ng/mg) (third column, figure 3). The sensitivity of urine CCR was 80%; 12 of 15 exposed children were consistently designated as exposed to ETS using urine CCR at the 30 ng/mg criterion level. Thus, for 24 children (12 exposed and 12 unexposed), accuracy of exposure categorization using urine cotinine was excellent at the 30 ng/mg criterion level. Only seven of 136 (5%) urine specimens tested from these children resulted in misclassification of exposure status. The remaining three exposed children were misclassified as not-exposed by 13 of 18 (72%) urine cotinine determinations using the 30 ng/mg CCR criterion. Two-day average home air nicotine levels in the homes of these three children were 1.45, 3.85, and 3.40 $\mu g/m^3$. Home air nicotine averaged 2.90 $\mu g/m^3$ for the three children not classified as exposed by urine cotinine compared to 3.95 $\mu g/m^3$ for the 12 children whose exposure status was consistently correctly identified by urine cotinine determinations.

The analyses that follow were performed with and without the data from the three exposed children with consistently low urine CCRs. Exclusion of data of exposed children with low CCRs did not alter the pattern of any analytic result; data shown are for all exposed children.

Correlation of Home Air Nicotine and Urine Cotinine Levels

Among ETS-exposed children, there was a significant positive correlation between the average concentration of nicotine in home air over the 2 days of monitoring and the average logarithm of urine CCR on the mornings after the 2 evenings of home air monitoring. In figure 4, average CCRs are plotted in relation to average home air nicotine levels for each of the 15 exposed children. The Pearson correlation for this set of data was $r \approx 0.68$; $p = 0.006$. The regression equation for the linear correlation is given in the figure legend.

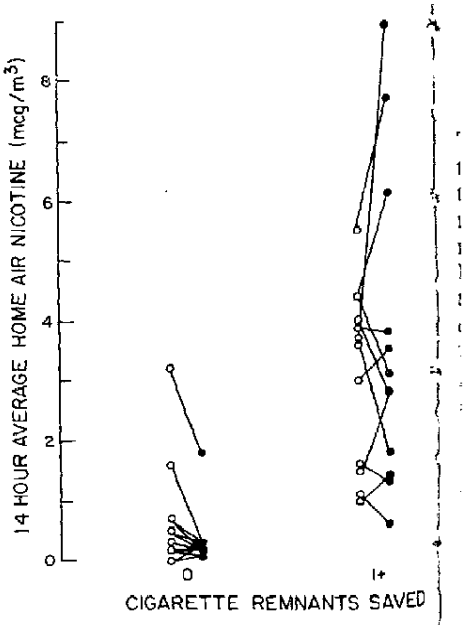

Fig. 2. Distribution of average home air nicotine concentrations for homes where cigarette remnants were and were not saved; measurements obtained on two consecutive days per home.

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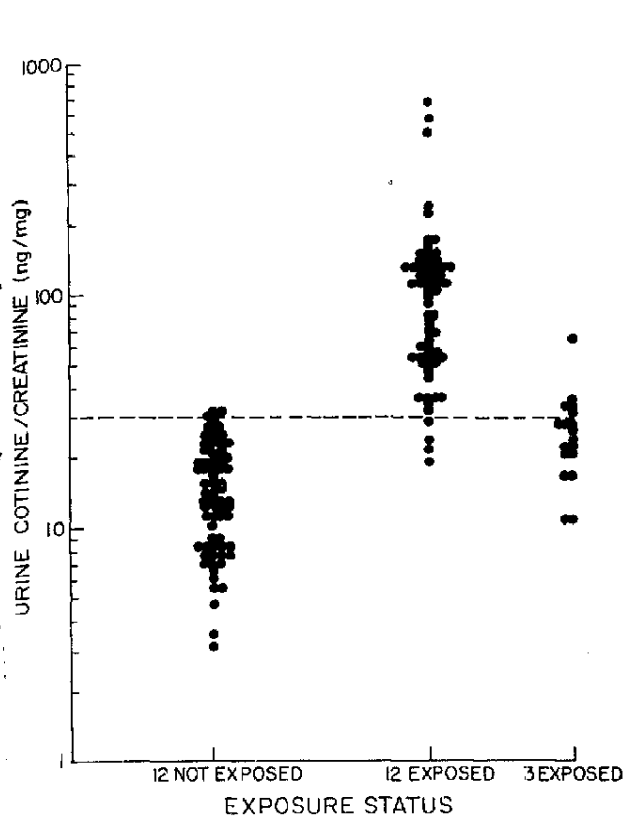

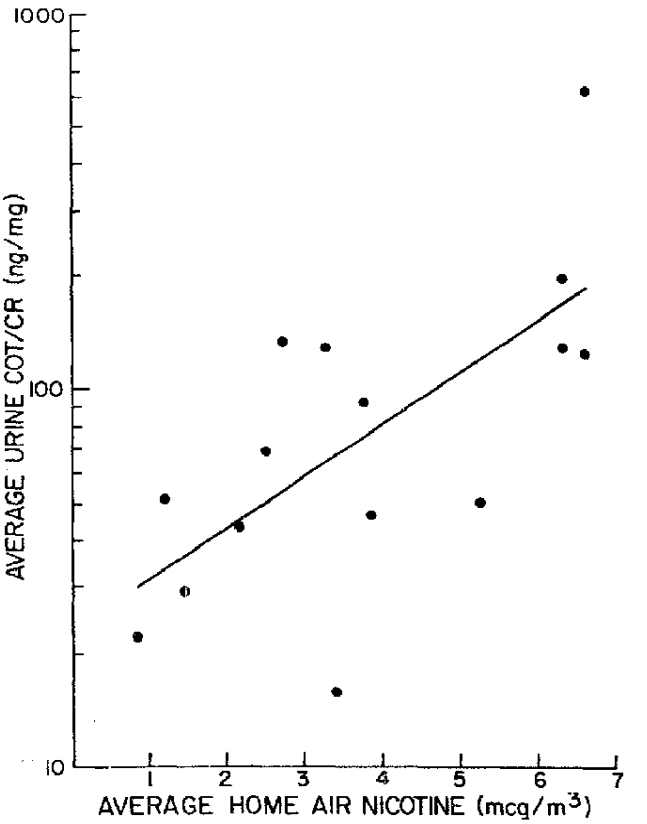

Fig. 3 (left). Distribution of all midmorning urine cotinine determinations from: column 1, 12 unexposed children (geometric mean CCR, 13.7 ng/mg; SEM, 2.3 ng/mg); column 2, 12 exposed children consistently classified as exposed with the CCR (geometric mean CCR, 86.5 ng/mg; SEM, 7.8 ng/mg); and column 3, three exposed children consistently classified as unexposed by urine CCR (geometric mean CCR, 24.5 ng/mg; SEM, 2.5 ng/mg).

Fig. 4 (right). Correlation between average home air nicotine concentrations for two monitoring days and average natural logarithm of urine CCR on the mornings after the two monitoring days for 15 ETS-exposed children. The equation for the regression line is: Ln CCR = 3.13 (SEM, 0.40) + 0.315 (SEM, 0.10) · average home air nicotine. ($F = 11.0$; $p = 0.006$.)

Observations Relevant to the Use of Urine Cotinine Determinations in Epidemiologic Studies

The urine collection protocol allowed us to examine whether time of urine collection during the day influenced urine cotinine levels. The means of $\log_e$ CCR did not differ significantly by sampling time. Furthermore, individual children maintained their absolute and relative levels of cotinine excretion throughout the two 24-h study intervals. In exposed children, rank correlations of sequential measurements ranged from $0.68 < r < 0.93$; $0.008 < p < 0.03$. There was no evidence that routine evening ETS exposures in children exposed chronically to ETS had acute effects on urine cotinine levels. For unexposed children, rank correlations of sequential urine CCRs were more variable, but as indicated previously the great majority of urine specimens (48 of 51 = 94%) had CCRs less than 30 ng/mg.

We then examined the stability of repeated urine cotinine/creatinine determinations over a 1-month time period. Urine specimens were collected while the children were at the day care center on each of four consecutive Thursdays. The data of 14 exposed children are shown in figure 5; one exposed child was absent frequently from the Center during the 1-month observation period. In exposed children, absolute and relative levels of urine cotinine were very stable over time. Rank correlations for sequential urine CCRs were all $r > 0.88$; $p = 0.0001$.

Discussion

Measurement of concentrations of cotinine in body fluids has been established as a useful method for identifying active smokers, persons exposed passively to environmental tobacco smoke, and neonates exposed to nicotine and cotinine in utero (1-13). Although active smokers can be distinguished readily from passive smokers with cotinine assays, these tests appear less accurate in distinguishing persons who are routinely exposed passively to ETS from those who are sporadically exposed. Furthermore, our knowledge of the usefulness of cotinine determinations for ranking the intensity of passive smoke exposure in populations of individuals is fragmentary. Most investigators who have attempted to examine this question have relied on questionnaire data to provide estimates of the intensity of ETS exposure in study populations. In the present work, we employed direct assays of home air nicotine concentrations to estimate the intensity of home nicotine exposure for study children.

The results of this study provide a firmer foundation for use of the CCR in urine as a biologic marker of ETS exposure in epidemiologic studies involving young children. Twelve of 15 preschool children routinely exposed passively to tobacco in the home had urine CCRs that were consistently > 30 ng/mg, whereas 12 of 12 children without home ETS exposure consistently had CCRs ⩽ 30 ng/mg. Three exposed children (20%) had urine

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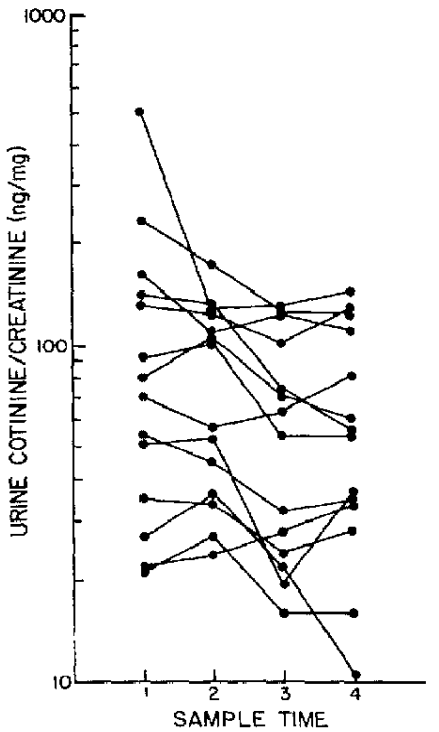

Fig. 5. Distribution of four consecutive weekly urine CCRs obtained from 14 ETS-exposed children. Rank correlations for sequential measurements are all greater than $r = 0.88$; $p < 0.0001$.

CCRs that were consistently in the upper range of values observed in unexposed children. Average home air nicotine levels were lower for these children than for exposed children with higher urine CCRs, but the difference was not statistically significant.

The correlation between average home air nicotine concentrations and the average $\log_e$ urine CCR among exposed children was $r = 0.68$, a significant association ($p = 0.006$). It is within the exposed group that a correlation must exist for cotinine to be a useful tool for assessing intensity of ETS exposure. The magnitude of correlation between air nicotine levels and urine CCR is dependent on the extent to which air sampling for nicotine in the home environment accurately reflected personal nicotine exposure and on the magnitude of individual differences in nicotine/cotinine metabolism for children with similar levels of environmental nicotine exposure. The degree of correlation between average home air nicotine concentrations and urine CCR observed in this study suggests that relative intensity of nicotine, and thus ETS, exposure can be estimated with a reasonable degree of accuracy using urine CCR.

Urine CCRs did not differ significantly by time of collection during the day. There was no evidence that usual evening ETS exposures altered urinary cotinine levels acutely. Furthermore, there was no evidence that short-term, high-level exposures, such as those occurring in closed automobiles, had a measurable impact on urine cotinine levels. Thus, for an individual child, any urine cotinine measured during a 24-h period was representative of the three others examined. Similarly, urinary CCRs were remarkably stable over a 1-month observation interval. Again, any single urine cotinine level was representative of the three others measured during the month. Relative urine CCR would have been assigned correctly using a single determination.

This study was conducted in a group of children who attended day care regularly. Urine cotinine levels might have been higher in young children who were exposed throughout the day to a smoking caregiver. The degree of overlap in urine CCR between some exposed and unexposed children might have been reduced in those circumstances. On the other hand, the study was conducted in the winter, when home ventilation rates are low compared to warm weather months. Performance of the study during the summer months could have reduced the accuracy of urine CCR as an exposure indicator.

These findings can be compared with those of other workers who have used salivary, serum, and urine cotinine determinations as indicators of passive smoking in children. Jarvis and coworkers (6) studied salivary cotinine levels in 569 nonsmoking English school children between 11 and 16 yr of age. Mean salivary cotinine concentrations were significantly higher in children with smoking parents than in children with nonsmoking parents. When both parents smoked, mean salivary cotinine levels were higher than when a single parent smoked. However, approximately 20% of children without smoking parents had salivary cotinine levels of 0.6 ng/ml or higher, whereas 20 to 30% of children with one smoking parent had levels below 0.6 ng/ml, indicating the potential for misclassification of exposure status. Coultas and colleagues (13) studied salivary cotinine levels in a population-based sample of adults and children in New Mexico. Salivary cotinine was detected in 38% of children less than 5 yr of age who lived in homes where no cigarettes were smoked. Conversely, 26% of children who lived in homes with smokers had no detectable salivary cotinine. In-home ETS exposure status of 33% of study subjects was misclassified using salivary cotinine determinations. These findings are comparable to those of Jarvis.

Pattishall and associates (9) studied serum cotinine levels in infants who attended the Frank Porter Graham Center, the site of the present study. Sera were obtained from 38 children at approximately 9 months of age; 20 children were identified by questionnaire as living in homes with smokers. Overall, there was a significant difference in the prevalence and average concentrations of cotinine in sera from exposed and unexposed children. However, cotinine was not detected in sera of 20% of children living in homes with smokers, while cotinine was demonstrated in sera of 26% of children living in homes without smokers. The accuracy of exposure assessment using serum cotinine was similar to that obtained by investigators studying salivary cotinine as a marker of ETS exposure in children.

Greenberg and colleagues (4) examined urinary and salivary cotinine levels in 46 infants: 28 with a history of passive tobacco smoke exposure and 18 with no exposure history. Urinary cotinine excretion distinguished exposed and unexposed children more reliably than did salivary cotinine determinations. Urine CCRs were significantly correlated with exposure dose estimated by questionnaire, when exposed and unexposed children were included in the analysis. When the correlation analysis was restricted to exposed children, no evidence of a dose-response relationship between reported maternal smoking intensity and infant cotinine excretion was observed (personal communication).

In a pilot study for the work presented here, we compared the sensitivity and specificity of serum cotinine concentrations and urine CCRs as correlates of home ETS exposure that had been documented by cigarette butt collections and home air nicotine monitoring. Cotinine was detected in sera of 16 of 26 (62%) children with home ETS exposure and in the sera of three of 20 (15%) children without home ETS exposure. Urine CCRs were more sensitive (21 of 26 = 81%) and more specific (18 of 20 = 90%) than serum cotinine levels for determining exposure status. For this reason, we focused our efforts on urine CCRs in the present study.

Determination of CCRs in urine samples is a reasonably accurate method for

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identifying passive tobacco smoke exposure in young children. Because some exposed children have relatively low levels of cotinine in urine, cotinine determinations should probably be used in conjunction with questionnaire data regarding ETS exposure. Data presented here indicate that urinary CCRs are related quantitatively to the intensity of home nicotine exposure among ETS-exposed children. This and the relative stability of urine CCR over time support use of the assay in epidemiologic studies of ETS exposure in young children.

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